

## Interactions between *Phytophthora Infestans* and Potato Host

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The white potato (*Solanum tuberosum*) was unknown outside of South America until the sixteenth century, but by the nineteenth century, it was widely cultivated in the British Isles, Europe, Russia, and the United States. Ireland, in particular, shifted its agricultural emphasis from cereal grains to the more productive potato with such success that the population of Ireland increased considerably to about 7 million by 1844. In 1845, climatic conditions and widespread growth of the potato in Ireland permitted catastrophic field infection by *Phytophthora infestans* and resulted in the loss of nearly the entire potato crop in that year and the subsequent year. The results for Ireland were devastating; over one million people died of starvation or diseases associated with malnutrition, and over one million more emigrated. *P. infestans*, the pathogen responsible for potato late blight, remains the most significant fungal parasite of the potato.

### The Nature of Late Blight

The symptoms of late blight on the potato plant are visible first as dark brown patches on the leaflets, usually near the margin. On the leaflets of varieties that are incompatible with the infecting race of *P. infestans*, the necrosis is restricted to small spots or flecks that do not enlarge. In a susceptible interaction, the necrotic areas expand, and white mold growth is usually visible along their edges on the underside of the leaflet. If weather conditions remain sufficiently damp, the infection can spread and destroy the entire haulm in a period of several weeks. During the course of progressive infection, spores are spread to other plants by wind, rain, or insects and transferred to the soil and the tubers of infected plants by rain.

The means by which late blight is perpetuated are shown in Fig. 1. In the vicinity of potato fields, elimination of cull piles containing blighted tubers is a well-accepted practice for

controlling the spread of infection from this source, and the selection of seed tubers as free from infection as possible is recognized as a necessity. One blighted seed tuber in 100,000 provides an adequate initial inoculum for a major epidemic (1). Control of the spread of infection between potato plants is dependent upon the use of fungicides such as the ethylenebisdithiocarbamates and the planting of potato varieties that have some degree of resistance. In the United States, the use of fungicides to prevent the spread of late blight generally is practiced in all areas except the western states where the summer climate is usually dry and uncondusive to the development of late blight. Under current management practices in the United States, late blight decreases the yield of potatoes at harvest by about 4%. Further losses are encountered in storage when tubers with minor blighted areas become mixed inadvertently with sound tubers. P. infestans does not generally spread to healthy tubers during storage, but the loci of infection provide footholds for secondary bacterial infections that spread to other tubers and cause significant losses (2).

#### Potato Resistance to Fungal Infection

The potato is immune to parasitization by the vast majority of fungi; there are, including P. infestans, only six or seven fungal parasites of the plant and tuber that are economically significant (2). The term immunity is used to denote complete and apparently permanent protection against a given parasite, whereas resistance denotes either incomplete or impermanent protection. Parallels have been drawn between immunity and resistance (1, 3), but Robinson (1) suggests that immunity is outside of the conceptual bounds of parasitism. Perhaps the major difficulty in conceptualizing immunity is that no one yet has formulated a research approach that might lead to a description of the circumstances responsible for the immune relationship of plants to fungi. Resistance, on the other hand, provides chemically and physically observable and measurable phenomena as a basis for research.

Resistance to fungal parasites commonly is divided into two general categories, horizontal resistance and vertical resistance. Horizontal resistance is said to provide a constant level (high, moderate, low, or nearly zero) of infectability with all existing and potential races of a given fungal species. From the constant level of infectability, the term and concept of horizontal resistance was formulated by van der Plank (4). It is believed that horizontal resistance usually has a polygenic origin in the host and that mechanisms responsible for horizontal resistance are likely to be numerous and complex. If this were not the usual case, minor genetic changes in a fungal parasite would be more likely to result in new races capable of parasitizing the host at higher levels.

Horizontal resistance can be viewed speculatively as a barrier of physical or chemical defenses which slow fungal invasion. Unavailability in the host of adequate essential nutrients required for fungal growth would constitute a passive form of horizontal resistance. The concept of horizontal resistance can also be extended to chemicals applied externally. The effectiveness of new fungicides is often temporary because the fungi produce new races unaffected by them. Other fungicides, such as Bordeaux Mixture, appear to have "horizontal properties" and maintain their effectiveness permanently, indicating that the fungi are unable to produce new races able to tolerate them. The inability of fungi to circumvent the toxicity of the "horizontal" dithiocarbamate fungicides, which inhibit twenty or more enzymes (5), is easily understood. The presence of a "natural horizontal fungicide" in host tissue would confer some degree of resistance, and there is evidence, cited later, that the potato tuber contains indigenous compounds with fungitoxic properties.

While potato varieties can exhibit varying degrees of horizontal resistance to P. infestans, most commercial varieties have (and most breeding programs are aimed at producing) vertical resistance (1). The term vertical resistance is derived from the observation that in some host-parasite interactions infection of a given host variety by different fungal races can occur at either a high level (susceptible or compatible interaction) or a very low level (resistant, incompatible, or hypersensitive interaction) (4). The terms compatible and incompatible are used in the remainder of this paper. Vertical resistance is often referred to as a gene-for-gene relationship between host and fungal parasite (1). Obviously, it is not the genes but the chemical and physical characteristics whose inheritance is genetically controlled that either match or do not match each other. Albersheim and Anderson-Prouty (6) extensively reviewed the evidence for the existence of genetically controlled recognition mechanisms that result in an incompatible response between fungal parasite and plant host. A formal classification scheme has been established to designate the genetic relationship between potato varieties and P. infestans races in terms of compatibility and incompatibility (7). In this widely accepted scheme, potato varieties are described as possessing vertical resistance genes designated as  $R_1$ ,  $R_2$ ,  $R_3$ , etc. A single potato variety may possess zero, one, or multiple resistance genes. P. infestans races are described as possessing pathogenicity genes designated as  $v_1$ ,  $v_2$ ,  $v_3$ , etc. (or as race 1, race 2, race 3, etc.), and a single race may possess zero, one, or multiple pathogenicity genes. A potato variety having the single resistance gene  $R_2$  interacts in a compatible manner with P. infestans races with the  $v_2$  gene and in an incompatible manner with all other races. Such a scheme has great utility as a classification system, but its empirical nature must be kept in mind. The designations used have no real meaning apart from the observed interactions between host and parasite.

Not all investigators believe that resistance can be neatly categorized as either horizontal or vertical. Also, both types of resistance may exist simultaneously in a single cultivar, although strong vertical resistance usually tends to mask horizontal resistance.

#### Native Properties Related to Resistance

The potato plant and tuber have native physical and chemical properties related to resistance. The epidermis of the foliar portion of the plant and the skin of the tuber present physical barriers to fungal and bacterial infection. A moist environment is required to permit spore germination and fungal invasion across these primary defenses. The stomata of the leaflets and the lenticels of the tuber represent weak points in the primary defenses, but wounding provides a more immediate foothold for fungal invasion.

As described by Kuć and Currier (8), a number of indigenous compounds which have some degree of fungitoxicity are found at higher levels in the outer few millimeters of the tuber. These compounds are chlorogenic acid, caffeic acid, glycoalkaloids, and scopolin. The levels of chlorogenic acid (9), caffeic acid (10), and glycoalkaloids (11, 12) increase in mechanically wounded tuber tissue, thus adding, perhaps, some degree of protection in an otherwise vulnerable situation. Rapid suberization followed by formation of a wound periderm in as little as 24 hr (2) is probably more important as a defense.

Scopolin and its aglycone scopoletin are normally present in potato tubers. The scopoletin content of tubers varies with the physiological state, being highest in newly-harvested, dormant tubers (13). Mechanical disruption of tuber tissue does not increase the concentration of scopolin, but infection by P. infestans, other fungi, bacteria, and viruses causes significant increases (14, 15).

#### Compounds Produced in Infected Potato Tissue

The incompatible interaction of P. infestans with potato tissue leads to the production of a number of compounds that are not native to the host. Studies have been carried out predominantly with tuber tissue disrupted by slicing prior to inoculation and care must be exercised in attempting to extrapolate such findings to events that may transpire in intact foliar tissue during field infection. Zacharius et al. (16) have shown that tuber tissue may not even provide a corresponding compatible or incompatible response when compared to the leaflets of the same potato variety.

The terpenoid structures that have been isolated and characterized as products of an incompatible interaction between P. infestans and potato tuber tissue are shown in Fig. 2. Tomiyama

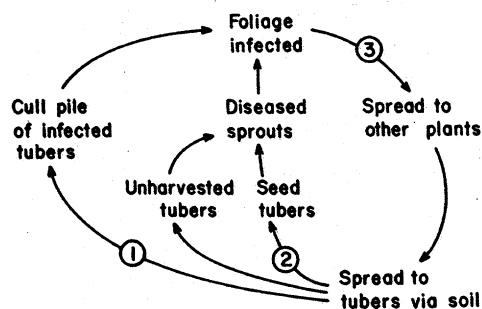


Figure 1. Cycle of *Phytophthora infestans* infection of *Solanum tuberosum*. Points at which the spread of infection may be disrupted effectively are indicated at 1, 2, and 3.

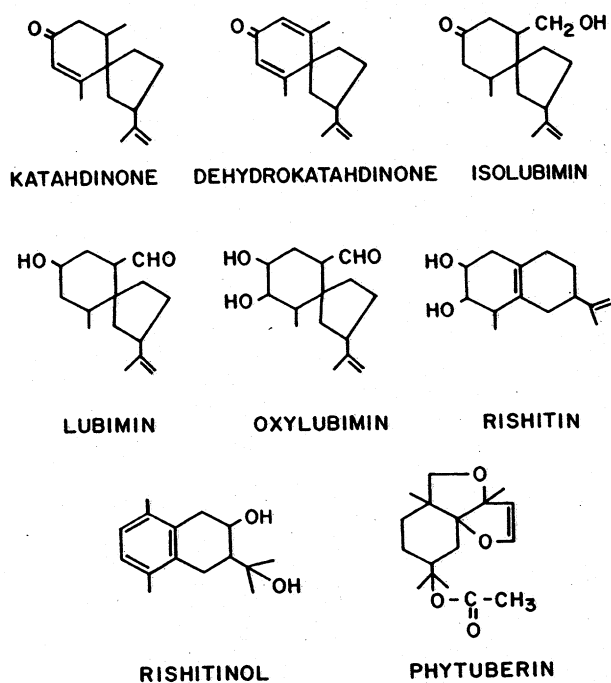


Figure 2. Compounds produced by the potato tuber host in response to infection. Most of these compounds have demonstrated fungitoxic properties and are often referred to as phytoalexins.

et al. isolated rishitin from infected Rishiri variety potatoes (17), and Katsui et al. characterized this compound (18). Katsui et al. (19, 20) isolated and characterized rishitinol. Varns et al. (21) isolated phytuberin, and Hughes and Coxon (22) established its structure. Metlitskii et al. (23) first isolated lubimin from a Soviet potato variety, Lyubimets, and Stoessl et al. (24) and Katsui et al. (25) determined its structure. Katsui et al. (25) isolated and identified oxylubimin; Kalan and Osman (26), isolubimin. Investigators in England and in the U.S. Department of Agriculture (27) simultaneously isolated and identified katahdinone and dehydrokatahdinone. The trivial name, katahdinone, was derived from the Katahdin potato variety from which the compound was isolated in the United States.

The use of potato tuber slices in the above studies is advantageous in that many potato cells are infected, inoculated slices can be incubated under controlled conditions, and the production of terpenoids is high per unit mass of potato tissue. The production of compounds in the incompatible interaction in intact foliar tissue is more difficult to study because the infection is restricted to very small areas. Metlitskii et al. reported lubimin production in potato leaflets inoculated with P. infestans (28). This suggests, at least, that terpenoid production may not be peculiar to tuber tissue.

While the above terpenoids have been isolated from incompatible interactions between P. infestans and potato tuber tissue, this is not the exclusive mechanism for their production. Zacharius et al. (29) reported that rishitin was produced in both a compatible interaction and an incompatible interaction at a similar rate during the first and second days following inoculation. Other reports document the production of rishitin, phytuberin, and lubimin in potato tuber tissue invaded by Erwinia carotovora var. atroseptica (30, 31, 32). The formation of terpenoids in potato tuber tissue chemically treated with NaF was reported by Metlitskii et al. (33), but this experiment could not be duplicated by Zacharius et al. (34).

In addition to the production of terpenoids, detectable changes in the soluble protein patterns of potato tissue interacting incompatibly with P. infestans have been reported. In infected tuber tissue, Tomiyama and Stahman found an increased number of electrophoretically separable protein bands (35). Yamamoto and Konno observed a new protein in leaflets of the potato plant 6 hr after inoculation with an incompatible race of P. infestans (36). It is possible that the incompatible interaction results in the induction of enzymes not normally present in the tuber and that such enzymes are the new, observable proteins. Further work is needed to determine the role, if any, of such proteins in resistance.

## Elicitors

Cell-free fungal preparations that initiate an incompatible response in host tissue have been referred to as elicitors or inducers. Sonication of *P. infestans* mycelia yields cell-free, autoclavable preparations that initiate a typical incompatible response, including terpenoid production and necrosis, when applied to potato tuber slices. Varns et al. (37) found that sonicates of both compatible and incompatible races of *P. infestans* produced an incompatible response when applied to tuber tissue. Glucans isolated from *Phytophthora megasperma* var. *sojae*, a fungal parasite of the soy bean plant, were reported by Ayers et al. to initiate an incompatible response in host tissues (38).

Albersheim and Anderson-Prouty suggested that elicitors are carbohydrate-containing molecules for which receptor sites exist on the plasma membrane of host cells (6). They further conjectured that the specificity of the receptor site for its "matching" carbohydrate elicitor may be the basis for incompatible interactions as the phenotypic expression of vertical resistance. In other words, receptor sites and elicitors may represent the genetically determined primary recognition factor for a potato variety and an incompatible race of *P. infestans*.

In a recent study, Kota and Stelzig demonstrated that preparations which act as elicitors caused membrane depolarization of potato petiole cells in less than 1 min following exposure (39). These preparations were a sonicate, a  $\beta$ -1,3-glucan, and a lipopolysaccharide (all obtained from *P. infestans*) and a preparation from *P. megasperma*. Among eight other carbohydrates which are not elicitors, only pectin produced depolarization. This report is supportive of the elicitor theory of Albersheim and Anderson-Prouty (6) and the mechanism of action of fungal phytotoxins advanced by Strobel (40).

In contrast to the theory of Albersheim and Anderson-Prouty (6), Ward and Stoessl proposed that a recognition mechanism (or incompatibility suppression mechanism) is involved in an interaction between molecules produced by the fungus and the compatible host (41). Metlitskii et al. added another dimension to speculation about the incompatible interaction by proposing that the potato host produces compounds which induce the formation of compounds by *P. infestans* which in turn induce the incompatible interaction in the host cell (42). Keen referred to the elicitor phenomenon as a derepression of the production of an antifungal compound in the soy bean host cell (43). More work obviously is needed to determine the nature of the primary events that determine compatibility or incompatibility between host and fungal parasite.

### The Phytoalexin Theory

Potato tuber tissue inoculated with an incompatible race of *P. infestans* was reported to display an incompatible interaction when later inoculated with a normally compatible race (44). This observation led to the "phytoalexin theory" (45), which holds that fungitoxic compounds, phytoalexins, are produced by host cells as a result of invasion by an incompatible fungus. Kuć and Currier (8) recently reviewed phytoalexin studies, most of which were conducted with members of the families Solanaceae (mainly the potato) and Leguminosae.

For the potato, the compounds shown in Fig. 2 are often referred to as phytoalexins and most of them have demonstrated fungitoxic properties. The fungitoxicity of rishitin, phytuberin, lubimin, katahdinone, and dehydrokatahdinone was tested against mycelial growth of *P. infestans* by Beczner and Érsek (46). At levels of 25 to 100 ppm in solid media in Petri dishes, all of the compounds exhibited moderate to strong inhibitory effects toward the further spread of 7-day-old cultures of *P. infestans* transferred to the media. Ward et al. (47) reported similar inhibition of mycelial growth with somewhat lower levels of rishitin. Germination of *P. infestans* zoospores was also inhibited by similarly low amounts of phytuberin (48), lubimin (49), and rishitin (50, 51). Since the levels of phytoalexins able to inhibit pathogen growth *in vitro* are attained in many incompatible interactions, investigators have suggested that phytoalexins play an important role in inhibiting fungal invasion (6, 52-57). However, ultrastructural studies (cited later) indicate observable differences between the compatible and incompatible interactions well in advance of the production of detectable levels of phytoalexins. This does not disprove the theory, since no one has yet determined the time of appearance or concentration of phytoalexins in the microenvironment of the individual host cell. Conversely, the proof of the theory would rest in determining that phytoalexins are present in sufficient quantities early enough in the infected, incompatible cell to inhibit fungal invasion and in identifying the mechanism of inhibition. Hohl and Stössel speculated that phytoalexins inhibit fungal glucanases necessary for both fungal growth and disruption of  $\beta$ -1,3-glucan barriers in the host cell (58).

### Ultrastructural Studies

The compatible and incompatible interactions between *P. infestans* and potato host cells have been studied by scanning electron microscopy and transmission electron microscopy to yield information complementary to biochemical studies.

Using a scanning electron microscope, Jones et al. (59) noted sharp boundaries between obviously infected cells near the surface and uninfected cells below the surface in the compatible interaction



of P. infestans with potato tuber tissue. In this interaction, hyphae penetrated intercellularly deep into the tissue where intact, live potato cells remained to support the colony with nutrients. A sharp boundary between necrotic cells and live cells was not observed in the incompatible interaction.

In electron microscopy studies of the infection of potato leaf cells, Shimony and Friend (60) observed penetration of host cells by P. infestans hyphae in both compatible and incompatible interactions. Although the rate of initial penetration did not differ, they reported visible ultrastructural differences between the compatible and incompatible interactions 7 hr after inoculation (with penetration occurring as late as 4-1/2 to 6-1/2 hr after inoculation). Between 9 and 12 hr, host cells surrounding the site of infection on the incompatible variety appeared to be dead and fungal hyphae were contained within the necrotic area; at 24 hr, severely damaged hyphae were observed; after 48 hr, there were no detectable living cells of either fungus or host in the small lesion. An observed feature of both the compatible and incompatible interactions was the formation of a sheath or encapsulation surrounding the intracellular hyphae. In a parallel ultrastructural study of the incompatible interaction between lettuce (Lactuca sativa) leaf tissue and a fungal parasite (Bremia lactucae), Maclean et al. (61) also observed that death of penetrated host cells preceded the cessation of growth and death of fungal cells by several hours.

Hohl and Stössel (58) also reported that fungal hyphae penetrated potato tuber host cells in both the compatible and incompatible interactions. The type of encapsulation described by Shimony and Friend (60) was observed in both incompatible and compatible interactions and was referred to as an extrahaustorial matrix. Hohl and Stössel, however, reported that the typical haustorium found in the incompatible tuber host cell differed from that of the compatible host cell by the presence of an additional entity, wall appositions, which surrounded and encased the haustorium and the extrahaustorial matrix. The encasement of haustoria has also been reported for the incompatible, but not the compatible, interaction of Uromyces phaseoli var. vignae with host cells of the cowpea (62). In two additional studies of a Phytophthora pathogen in compatible and incompatible interactions, the presence of wall appositions was not reported (63, 64).

In studies of leaf tissue of the same two potato varieties employed in tuber tissue studies by Hohl and Stössel (58), Hohl and Suter (65) found that haustoria in both the compatible and incompatible interactions were similar and were always surrounded by an extrahaustorial matrix and sometimes also by wall appositions. As a possible explanation for the marked ultrastructural differences in haustoria observed between the two types of tubers but not in the leaf tissues, the authors pointed out that the leaflets of the two varieties differ less in resistance than the corresponding tubers. Various types of fungal invasion of potato leaf cells

ranging from hyphae which penetrated cells and then emerged at another point to fully developed haustoria were observed by Hohl and Suter. Various degrees of haustorial encasement were also observed as shown in Fig. 3, and the authors suggested that host cell resistance may be related to the degree of encasement that takes place. They further noted that hyphae enter the leaf primarily through stomata but are also able to penetrate epidermal cells or hair cells. In the incompatible interaction, the parasite did not penetrate deeply into the host tissue and rarely developed sufficiently to produce sporangia.

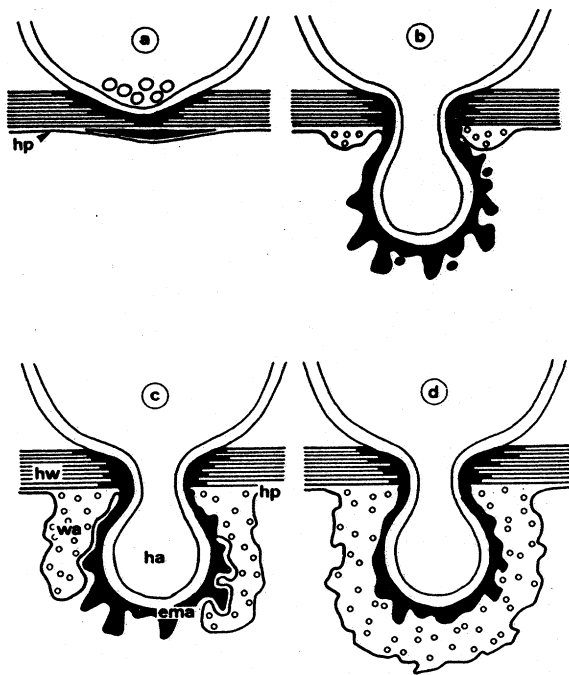
While ultrastructural studies of compatible and incompatible interactions of P. infestans with the potato host are not in complete agreement, common findings have emerged: cell penetration occurs in both circumstances; in the incompatible interaction, penetration occurs to a depth of very few cells followed by death of the host cells and later death of the fungal cells.

### Conclusion

Certain aspects of the incompatible interaction between P. infestans and the potato host seem to be reasonably well-documented. A number of compounds with demonstrated fungitoxicity are produced when tuber tissue is invaded, host cells become necrotic and fungal cells later die. Identical symptoms in tuber host cells can be initiated by the application of cell-free preparations of the fungus. However, the observed symptoms of incompatibility may be far removed from the primary event that triggers the sequence of known events. The fact that fungitoxic compounds are evolved is not complete proof that they are, indeed, the cause of incompatibility in the complex cell-cell interaction. Gene-for-gene interactions between host and parasite can be systematically tabulated, but only in the empirical terms of compatible or incompatible. The phenotypic mechanisms involved in interactions at the cell and molecular levels remain to be described. Elucidation of the molecular basis for the specificity exhibited in gene-for-gene, host-pathogen interactions is within reach and is an important objective.

Although valuable information has been gained through studies with tuber tissue, it appears that additional studies with foliar tissue should be emphasized. Phenomena observed in interactions of P. infestans with sliced or otherwise disrupted tuber tissue cannot be assumed to occur in foliar tissue. Since resistance to P. infestans must be expressed in the foliar portion of the potato plant in order to break the cycle of infection shown in Fig. 1, it is of primary importance to describe and understand resistance in terms of the leaflets rather than the tubers.

Great emphasis has been placed on understanding vertical resistance in the potato, while horizontal resistance has received comparatively little study. Since vertical resistance is temporary and effective only until a new race of P. infestans capable of



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Figure 3. Diagram of haustorial development of *Phytophthora infestans* on potato leaves. (a) Early penetration of host wall with incipient extrahaustorial matrix formation between host wall and host plasmalemma (hp). (b) Haustorium ensheathed by extrahaustorial matrix and collared by wall appositions at its neck. (c) Progressive encasement of haustorium (ha) by wall appositions (wa). The host plasma membrane (hp) may double-over on itself at the interface of the extrahaustorial matrix (ema) and the wall apposition. (d) Fully encased haustorium with compressed extrahaustorial matrix. Haustorial development may terminate at (b), (c), or (d), probably reflecting rising degrees of host cell resistance (65).

bypassing the resistance mechanism evolves, additional studies aimed at identifying the physical and chemical features of the potato that contribute to horizontal resistance seem in order.

Despite the extensive efforts that have been devoted to breeding potato varieties resistant to *P. infestans*, potato growers must rely heavily on the use of fungicides to control late blight. Environmental and economic considerations discourage the continued use of conventional fungicides and encourage further research to understand the chemical basis for potato plant resistance to *P. infestans*. Robinson (1), critical of the unholistic approach to disease resistance, pointed out that the disciplines involved have concentrated too heavily upon their particular interests. Effective teams that include plant breeders, plant pathologists, microscopists, and chemists will probably have the greatest impact in the further conceptualization of resistance and the practical application of the concepts to the development of varieties with improved resistance.

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